

EXHIBIT C4

2d ET Sm of June 28 to 1000 Q
 Cas 8 activation

RYT

		vol	Y	10Y
SK2N	.166 (5X)	30	.25	39 30
SK1E	.253 (5X)	30	.66	15.3 11.8
SK36	.141 .142 (5X)	60	.17	5.8 6.5
SK40	.234 (5X)	30	.57	17.6 13.6
SK42	.235 (5X)	30	.57	17.5 13.6

(A) Lo PH,
 no DIT, 15"

- RIPA/NPDC
 15", no DIT

(SNAP PROTEIN)

CL2N	.328 (5X)	.125 (5X)	.07	$\boxed{29X} = 1.75X$
CL2E	.329 (5X)	.135 (5X)	.11	$\boxed{29X} = 15.9X = 1.75X$

DM

SK2N	.332 (2X)	2.5	3.9
SK1E	.450 (2X)	3.9	8.6
SK36	.191 (2X)	.92	10.8
SK40	.404 (2X)	3.4	3.0
SK42	.571 (2X)	3	3.3

CL2N	.328 (5X)	1.0	10
CL2E	.329 (5X)	1.0	10

Ge1 (3) Cas 8 PM 10X

(M) SK2N	1E	36	40	42	(M) CL2N	CL2E (M)
(1:2) 7.8	5.2	21.6	6	6.6	20	20

Ge1C4 Cas 8 C4T 10X SK, 1.75X CL

SK2N	1E	36	40	42	(M) CL2N	CL2E
(1:2) 46.8	18.4	69.6	21.1	21	(1:2) 3.5	3.5
20	50	50	50	50		

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Results

At 36 hrs processing of Figure 8 is seen in melanoma cells

Cyt fraction $\left[\begin{array}{l} - \sim 43 \text{ kD} \\ - 24 \text{ kD} \text{ * also appears @ 42 hrs but not 40 hrs} \\ - \sim 20 \text{ kD} \\ - \sim 18 \text{ kD} \end{array} \right.$

In addition a large band $\sim 80 \text{ kD}$ is induced @ the plasma membrane, the 24 kD fragment is shifted \uparrow in the fraction

- No active S processing seen in melanocytes

Results

- ET-1 activates active S transiently 36 hrs seems maximal though hasn't peaked at time points between 24 & 36 hrs

- Kinetics of activation may be different in melanocytes and/or may need to be grown in PDGF post (the cells were not very healthy and were then directly from PDGF).